Potential of mesenchymal stromal cells for improving islet transplantation outcomes

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Abstract

Allogeneic islet transplantation as a therapy for Type 1 Diabetes (T1D) is restricted by the limited availability of donor islets, loss of functional islets during pre-transplantation culture *in vitro* and further extensive loss during the immediate post-transplantation period when islet function and survival is compromised by the hypoxic, inflammatory host environment. In the longer term pathogenic T cell responses drive autoimmunity and chronic allograft rejection. Experimental studies have demonstrated that Mesenchymal Stromal Cells (MSCs) have significant potential to improve the outcomes of clinical islet transplantation. This review explores the potential for MSCs and their 'secretome' to influence donor islet cell function and survival, as well as the host niche. We discuss the possibility of harnessing the therapeutic benefits of MSCs in a cell-free strategy to offer a well-defined, cell-free approach to improve the outcomes of clinical islet transplantation.

Introduction

Islet transplantation

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which insulin-secreting pancreatic β-cells are selectively destroyed. The consequent insulin deficiency results in dysregulation of metabolic control with hyperglycemia, hyperlipidemia and ketosis leading to a fatal outcome. People with T1DM have been treated by the administration of exogenous insulin since the first successful isolation of biologically active insulin in 1921. However, insulin therapy treats the symptoms of T1DM rather than offering a cure, and it has become clear over the decades that exogenous insulin often fails to maintain tight glycaemic control, and that the subsequent hyperglycemic excursions are responsible for the progressive development of a range of devastating side effects known as "secondary complications"[1]. Replacement of the damaged β-cells offers the potential for restoring physiological glycemic control and thus avoiding the development of secondary complications. However, pancreas transplantation is surgically invasive and associated with significant co-morbidity [2]. An alternative is to transplant only the endocrine component of the pancreas - the islets of Langerhans- which comprise 2-3 % of the total pancreas, and which can be isolated from the intact pancreas by collagenase digestion. Islet transplantation first became a viable therapeutic option with the publication of the landmark "Edmonton Protocol" in 2000 [3], which stimulated human islet transplantation programmes around the world, such that over 1,500 people with T1DM have now received islet grafts in 40 centres globally (https://citregistry.org/content/citr-9th-annual-report). Islet transplantation is a safe procedure with little or no co-morbidity [4] and the clinical outcomes have improved year-on-year [4,5]. Recent figures suggest that approximately 50 % of graft recipients remain insulin independent at 5 years and more, making islet transplantation as clinically effective as whole pancreas transplantation [6]. However, the wider adoption of islet transplantation as a therapeutic option is currently limited by a shortage of tissue donors and much effort is currently directed at generating functionally-competent substitutes for primary human islets to expand the available pool of graft material.

Islet β-cells are metabolically active and use glucose metabolism and ATP generation to maintain appropriate rates of insulin secretion for the prevailing extracellular glucose concentrations. Islets deteriorate rapidly during and after their isolation [7], losing 20-50 % of the β-cell mass within 24h of culture *in vitro*. The loss of β-cell function continues during the immediate post-transplantation period (24-72h) when up to 70 % of the graft function is lost because of deleterious responses of the transplanted β-cells to the hypoxic, inflammatory, immunogenic host environment [8]. Strategies which improve the functional survival of islets both before and after transplantation will improve the

outcome of individual grafts and enable the limited pool of donor islets to treat many more people with T1DM. One emerging strategy is the use of mesenchymal stromal cells (MSCs) to take advantage of their anti-inflammatory, immunoregulatory, angiogenic and regenerative properties.

Mesenchymal stromal cells and islet transplantation

MSCs are multipotent adult stromal progenitor cells located in the perivascular niche of most adult tissues, where they are involved in regeneration and repair in response to tissue ageing or damage. Importantly, MSCs can be isolated from their host tissues and maintained and expanded *in vitro*. There is no single specific marker for identifying MSCs, so the International Society for Cellular Therapy has specified three essential criteria to define cells as MSCs. First, cells must be plastic-adherent when maintained in standard culture conditions. Second, ≥ 95 % of cells must express CD105, CD73 and CD90 but not express the hematopoietic markers CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II. Finally, MSCs must be capable of differentiation into osteoblasts, adipocytes and chondroblasts *in vitro* [9].

There is accumulating evidence that MSCs can improve the outcomes of islet transplantation in experimental models. Thus, co-administration of MSCs improved islet graft function in syngeneic [11-12, 21], allogenic [13-16] and humanised mouse models [17] and in non-human primate models [18] of islet transplantation. A range of *in vitro* and *in vivo* studies suggest that MSCs exert direct effects on the islet β-cells to improve their post-transplantation function and survival, and also influence the host environment to supress inflammatory innate immune responses [19] and T-cell-dependent acquired immunity [20], and to enhance revascularisation of the graft [11-13,21].

Influencing the host environment

Isolated islets are metabolically fragile because of the stresses (mechanical, enzymatic), loss of extracellular matrix (ECM) [22] and ischaemia associated with the isolation process. These fragile islets are transplanted into a hostile inflammatory environment leading to further β-cell death [23]. The clinically–preferred route for graft delivery via the hepatic portal vein has the advantages of being simple, minimally-invasive and targeting insulin to the liver, but intravascular delivery triggers the instant blood mediated inflammatory response (IBMIR). This leads to thrombus formation around the islets and the initiation of an inflammatory cascade, both of which are deleterious to the islet graft [24]. In the longer term, auto- and allo-reactive T cell responses to transplanted islets contribute to graft attrition [20], and the metabolically-active β-cells are susceptible to anoxia during the revascularisation process [25].

Modulating the host immune response

MSCs can influence cells of the innate and adaptive immune systems and their co-transplantation at extrahepatic sites creates an immunosuppressive niche [14]. For example, MSCs influence macrophage phenotype by inducing a shift from pro- (M1) to anti-inflammatory (M2) macrophages (reviewed in [19]), which reduces graft neutrophil infiltration and inflammation during the immediate post-transplantation period. MSCs also have the potential to intercept the priming and amplification of autoreactive T cells. Thus, in T1DM MSCs can drive monocyte-derived dendritic cells (DCs) toward an immature, IL-10 producing regulatory phenotype via mechanisms dependent on IL-6, TGF-β and prostaglandin-E2 (PGE2) production [26]. More chronically, MSCs or multipotent adult progenitor cells (MAPCs [27]; off-the shelf clinical grade MSCs) influence cells of the acquired immune system to supress pathogenic T cell proliferation [13,28-31] and the production of proinflammatory cytokines, whilst favouring a more protective regulatory T cell response and altering the Th1/Th2/Th17 cytokine balance [15,20,32,33]. MSC co-transplantation prevents the Th17 immune response in a mouse allogeneic model of extrahepatic islet transplantation, but this effect was only observed when MSCs were co-delivered with the islets beneath the kidney capsule to create a local immune-privileged site, and not when the MSCs were injected systemically (intravenous) [14]. This has implications for delivering human islets via the clinically-preferred intraportal route because this will not facilitate the co-engraftment of islets and MSCs since their different sizes (~100µm and ~10µm diamater, respectively) will ensure that they lodge in different compartments of the hepatic microcirculation.

An alternative strategy is to identify the key MSC-derived soluble mediators which mediate the beneficial effects and use these in a “cell-free” therapeutic capacity. MSC-derived exosomes [26,34] and secreted factors have been shown to contribute to the immunosuppressive properties of MSCs (reviewed in [35]), including matrix metalloprotease 2 (MMP2), MMP9 [28], PGE2 [36,37], IDO [32], HGF, PDL-1, TGF-β, IL-4 and TSG-6. In addition, we have recently identified a range of MSC-secreted ligands for islet G-protein-coupled receptors (GPCRs; [38,39], including Annexin A1 (ANXA1) and chemokine (C-X-C motif) ligand (CXCL) 12, which can influence T-cell mediated immune responses. CXCL12 repels cytotoxic CD8+ T cells from infiltrating the islet graft whilst attracting protective regulatory T cells, and coating islets with CXCL12 delays allogeneic graft rejection [40]. Systemic administration of ANXA1 delays pro-inflammatory Th17 cell-mediated progression of autoimmune uveitis [41,42], a mechanism that may also be important in reducing Th17-mediated β-cell apoptosis in autoimmune T1DM and clinical islet transplantation.

Improving revascularisation

Transplanted islets are avascular during the immediate post transplantation period exposing β-cells to a hypoxic microenvironment [25] which can lead to ischaemic cell death, so enhancing graft revascularisation should improve transplantation outcomes. Numerous studies have demonstrated that co-transplantation of MSCs [11-13,21] or MAPCs [27] improves islet revascularisation via a range of pro-angiogenic factors [12,43]. For example, Cunha and colleagues [27] identified angiogenic factors expressed by MAPCs, including vascular endothelial cell growth factor (VEGF-A, -C, and -D), placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1) and IL-8, which are likely to contribute to enhanced revascularisation. Wnt signalling in MSCs promotes the secretion of pro-angiogenic proteins as well as matricellular proteins that generate a niche for islet regeneration [44], and MSCs secrete Wnt4 and Wnt5a [38]. However, these factors have opposing effects on islet endothelial cell survival (EC) with Wnt4 conferring protective effects and Wnt5 detrimental effects [45]. Notably, the protective effects of Wnt4 were also associated with reduced expression of vascular adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1), indicative of reduced inflammation in the ECs. Identifying and delivering MSC-derived molecules which enhance islet graft revascularisation should improve β-cell survival during the crucial immediate post-transplantation period.

Improving graft functional survival

Several experimental studies have shown that MSCs maintain functional β-cell mass during culture *in vitro* [46-48], with associated improvements in islet graft function *in vivo* [46,47], and it is now well established that MSCs have direct effects on β-cells to enhance their insulin secretory capacity [12,46-49] and protect them from cytokine-induced apoptosis [50]. Direct cell-cell contact plays a role in the capacity of MSCs to promote insulin secretory function [46,47,51], with N-cadherin interactions maintaining the function of encapsulated islets in diabetic mice [51]. It is well established that ECM components exert beneficial effects on the function and viability of islets. Both mouse and human MSCs lay down an ECM consisting mainly of collagen 3A1 and 4A1 *in vitro* [39,52] and MSC-derived ECM enhances islet insulin secretory function *in vitro* [39]. The precise mechanism through which islet-ECM interactions enhance islet insulin function is unclear but integrin receptors are likely to play a role. Integrins are a large family of αβ heterodimeric transmembrane glycoproteins which maintain cell adhesion and provide bidirectional signal transduction across the islet cell membrane. Islet-integrin-ECM binding has been linked to the activation of signalling cascades components implicated in improved islet function and survival, such as PKB/Akt and Erk1/2 [53]. More recently, it has been

shown that collagen 3, a common component of ECM, potentiates insulin secretion by activating β-cell GPCR 56 [54], which is the most abundantly expressed GPCR in mouse and human islets [55].

Soluble secretory products also play important roles in the beneficial effects of MSCs on islet function. [38]. Hypoxic preconditioning of human adipose-derived MSCs has been shown to alter their secretome, with associated improvements in human islet function and protection from cytokine-induced apoptosis [56]. We have demonstrated recently that pre-culturing islets with ANXA1 *in vitro* improved the functional quality of mouse and human islets [38,39] and the outcomes of subsequent experimental islet transplantation [38]. We have recently extended these studies to demonstrate that a defined 'cocktail' of MSC-derived molecules is more effective than ANXA1 alone. Thus, pre-culturing mouse islets with a cocktail of three MSC secretory products – ANXA1, SDF-1 and complement component C3a - induced persistent improvements in insulin secretion and protection from cytokine-induced apoptosis for up to 72h after the pre-incubation period (Rackham et al., under review), which covers the immediate post-transplantation period when graft survival is compromised [23]. These *in vitro* observations correlate with improved *in vivo* function of islets which were pre-cultured with the cocktail before implantation (Rackham et al., under review). Recent studies suggest that the persistent effects of ANXA1, following its removal from the culture media, may be due to the up-regulation of the formyl peptide receptor (FPR)2/ALX, PI3K and subsequent downstream pathways including activation of pro-survival Akt [57]. Restoration of Akt signalling in the heart and kidney has also been demonstrated following treatment with recombinant ANXA1, with associated reductions in the development of both cardiac and renal dysfunction, which is typically associated with the underlying diabetic phenotype [58]. Thus, MSCs and their 'secretome' maintain functional β-cell mass *in vitro* and support islet functional survival after transplantation, with the additional potential to reduce the development and progression of secondary microvascular complications.

Conclusion

There is convincing experimental evidence that MSCs improve the outcomes of experimental islet transplantation and therefore have the potential to improve human islet transplantation as a therapy for T1DM. It has become evident that MSCs secrete molecules which have beneficial effects on the islet graft and/or on the host environment. Defining a cocktail of MSC secretory factors which improves the functional survival of donor islets, enhances their revascularisation and reduces inflammatory and pathogenic T cell responses raises the possibility of harnessing the therapeutic benefits of MSCs in a cell-free strategy [38,59], avoiding the logistical, safety and regulatory concerns of including MSCs in clinical islet transplantation protocols. Schematic 1 shows three potential sites

for therapeutic intervention: pre-treating the donor islets with a cocktail of factors to improve subsequent functional survival; short-term co-delivery of defined factors to the site of islet engraftment, either by pre-coating the islets [40] or by incorporating the factors into polymeric islet nanocoatings [60]; and longer-term systemic administration of chosen factors. We suggest that, in combination, these approaches offer a well-defined, cell-free approach to improve the outcomes of clinical islet transplantation.

Conflicts of interest statement

Nothing declared

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Figure Legend

Figure 1: Schematic for proposed delivery of MSC-derived secretory products during the islet transplantation procedure. Based on experimental studies, we propose a tripartite strategy for using MSC secretory products to improve islet transplantation outcomes.

1. Preculture islets *in vitro* with a defined cocktail of soluble MSC-derived secretory products for 24-48h before transplantation to enhance subsequent graft function.
2. Co-delivery of defined MSC-derived secretory products with the islet graft will ensure localised effects on graft and host niche *in vivo,* during the early post-transplantation period (7-14 days post-transplantation).
3. Systemic delivery of MSC-derived secretory products will enable sustained effects of MSC-biotherapeutics *in vivo*.

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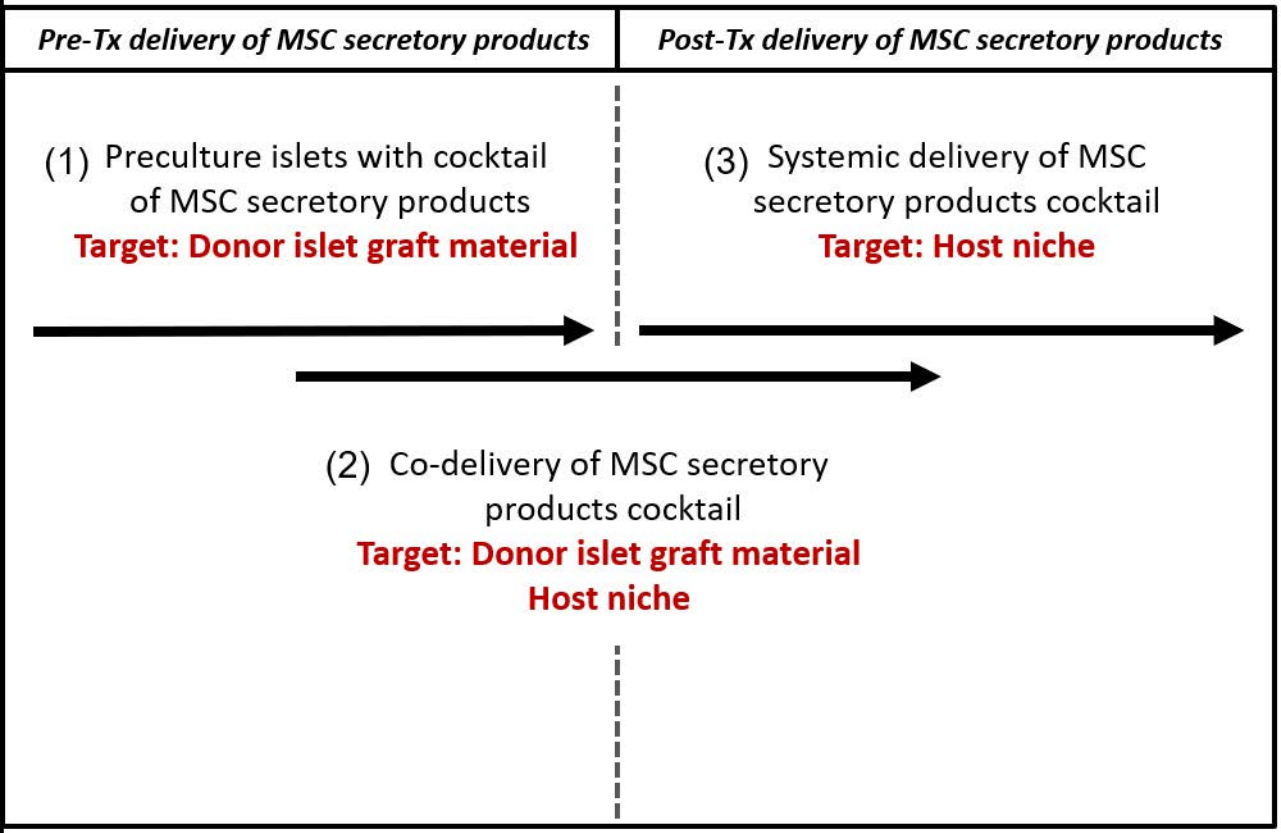
Pre-Tx delivery of MSC secretory products
Post-Tx delivery of MSC secretory products
(1) Preculture islets with cocktail of MSC secretory products
Target: Donor islet graft material
(3) Systemic delivery of MSC secretory products cocktail
Target: Host niche
(2) Co-delivery of MSC secretory products cocktail
Target: Donor islet graft material
Host niche